

Conference Vaccine Cell Substrates 2004

Diagnostics for TSE agents

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TSE Diagnostics

OVERVIEW

- > Post mortem diagnostics
 - > Histopathology (vacuolar changes)
 - > Immunohistochemistry (PrP^{Sc})
 - > EM/SAF (Electron Microscopy of Scrapie Associated Fibrils)
 - > Bioassay
 - > **Rapid tests (immunochemical detection of PrP^{Sc})**
- > Ante mortem diagnostics
 - > **PrP^{Sc} (conformation specific antibodies)**
 - > Surrogate markers
 - > Erythroid differentiation related factor (EDRF)
 - > Fatty acid binding protein (FABP)
 - > Cellular nucleic acids
 - > Cystatin C

TSE Diagnostics

RAPID TESTS

1999 EU Approved

- | | |
|---------------------------|-------|
| > Prionics®-Check WESTERN | WB |
| > Bio-Rad Platelia | ELISA |
| > Enfer BSE Test | ELISA |

2003 EU Approved

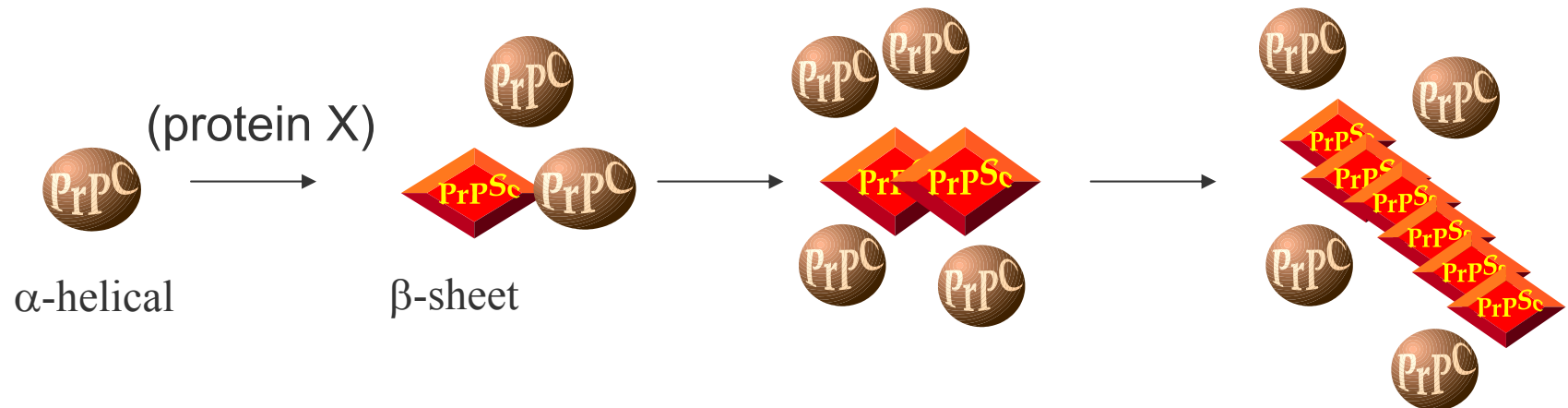
- | | |
|-----------------------|-------|
| > Prionics®-Check LIA | ELISA |
| > InPro CDI-5 | ELISA |

2004 EU Evaluation

- | | |
|-----------------------------|------------|
| > Prionics®-Check PrioSTRIP | Strip test |
| > Several new tests | |

Prion Biology

THE 'PROTEIN ONLY' THEORY



- > Prusiner (1982) 'Novel proteinaceous particles causing scrapie'
- > Nobel price, 1997

TSE Diagnostics

DETECTION PRINCIPLE

normal



▼ Proteinase K



BSE



▼ Proteinase K

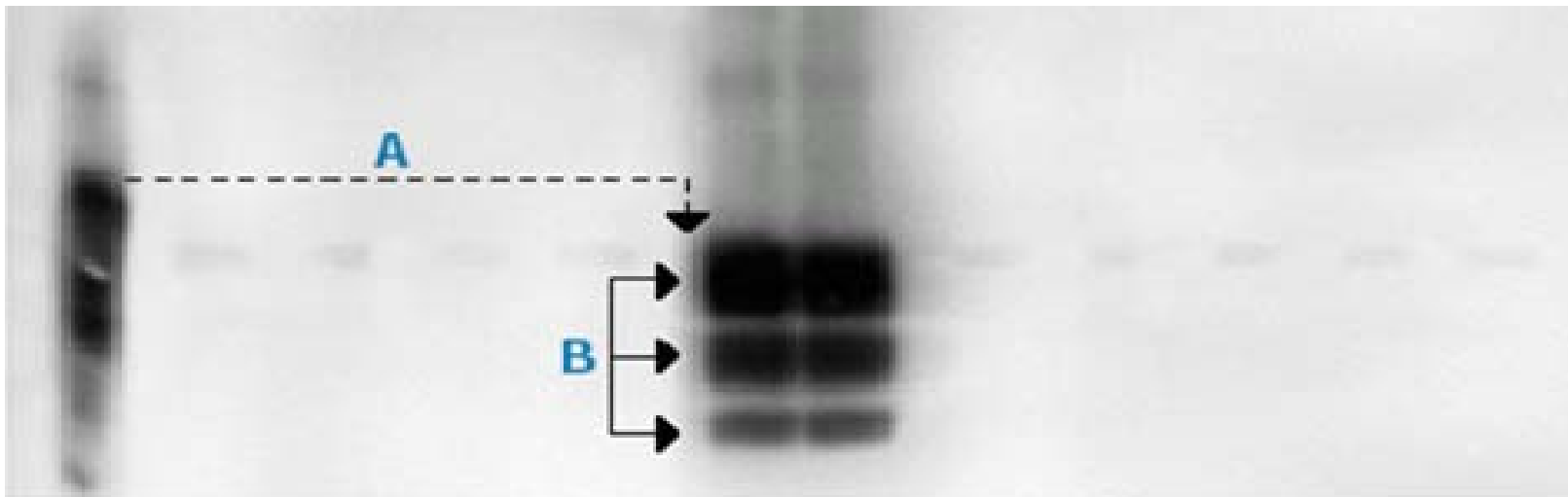


Prionics®-Check WESTERN RESULT

Control

BSE

Normal

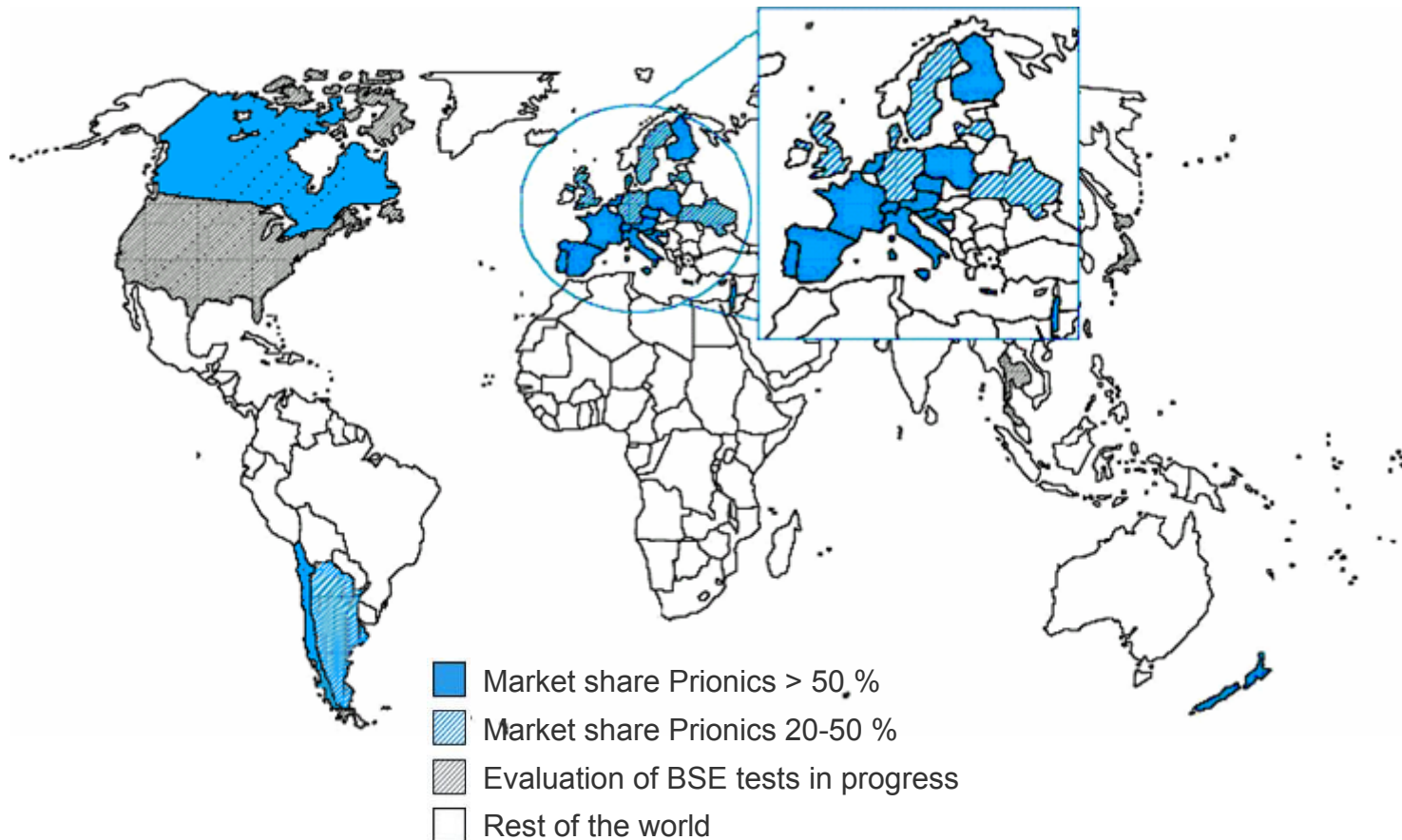


Immunological detection: Identification of PrP through presence of signal.

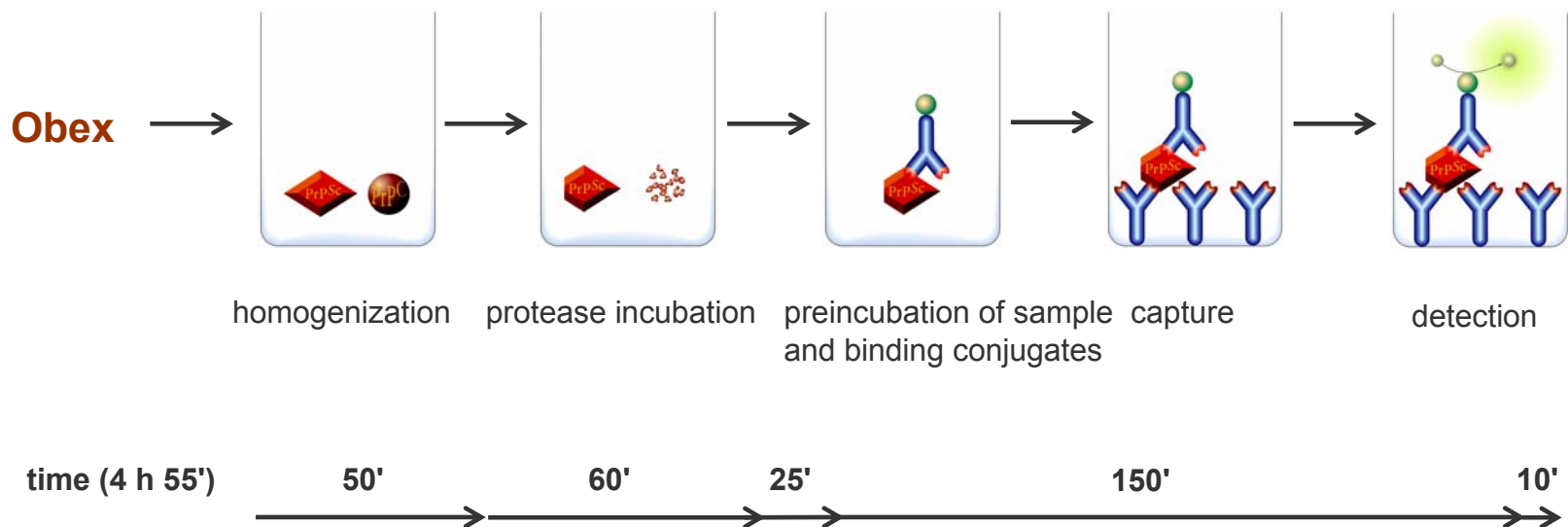
A Molecular weight: Absence of higher molecular bands serves as an internal control for complete digestion of PrP^C.

B Glycosylation Pattern: Characteristic band pattern confirms PrP^{Sc}, rather than putative contaminants, as the origin of signal.

Prionics®-Check WESTERN > 20 MIO TESTS: NO FALSE POSITIVES

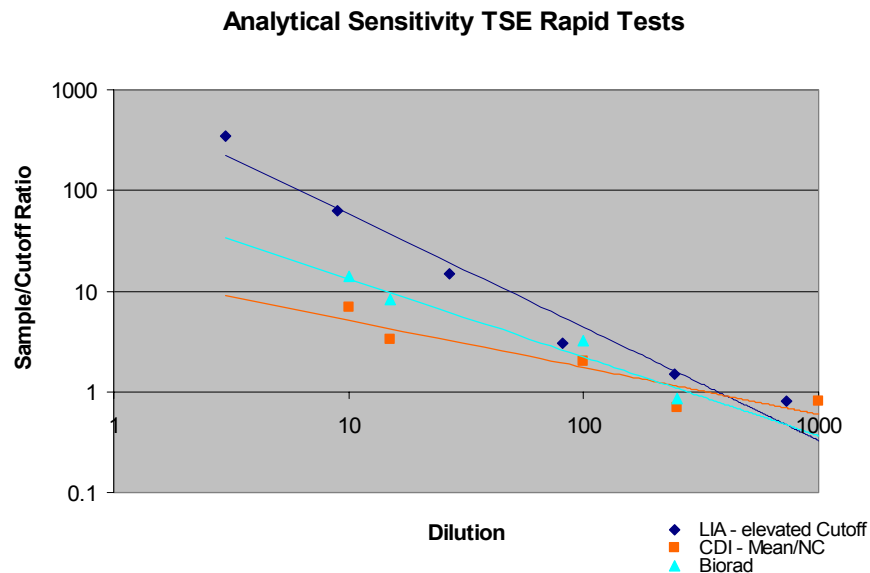


Prionics®-Check LIA TEST PRINCIPLE



2002 EU Field Trial ANALYTICAL SENSITIVITY

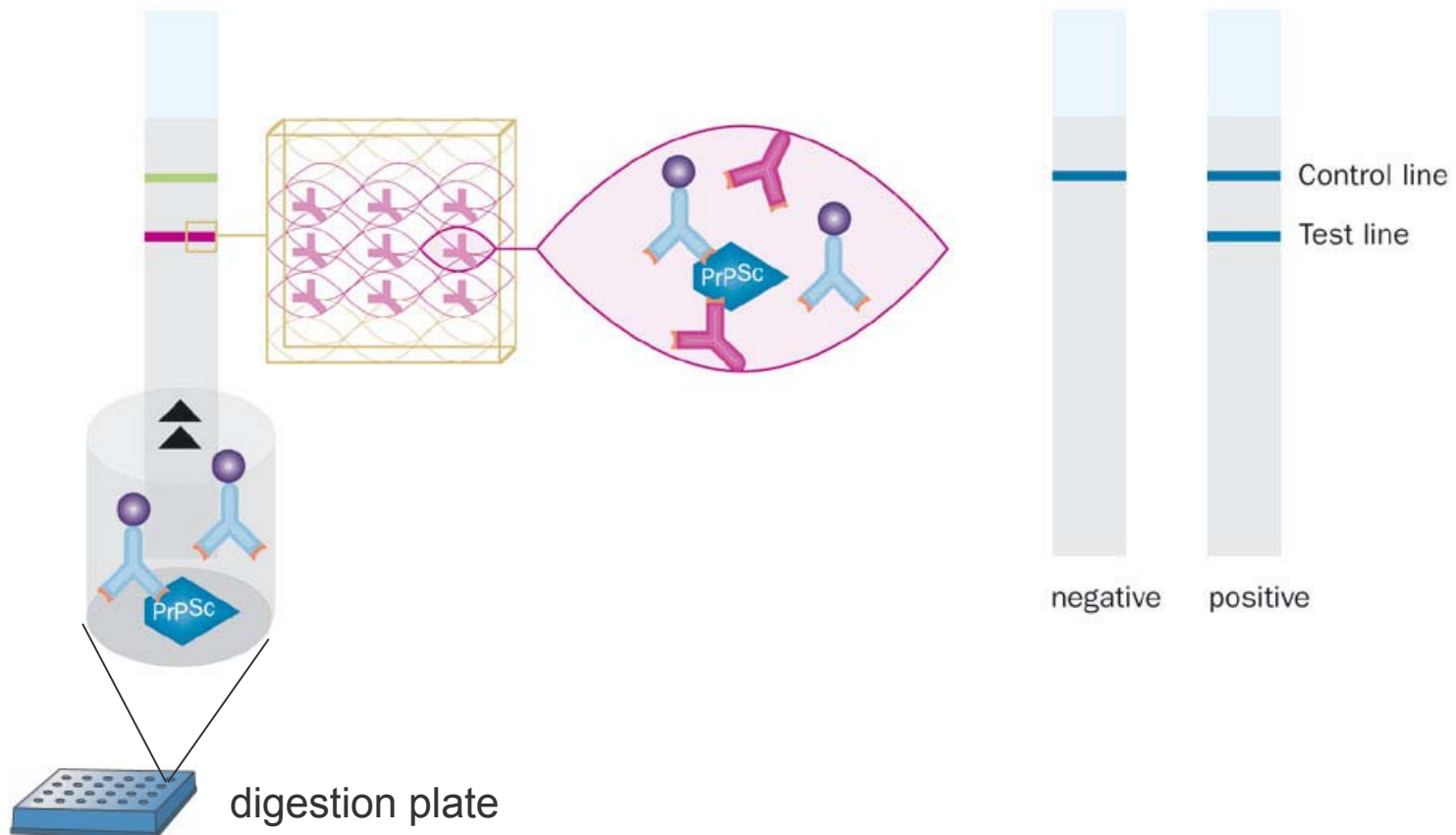
- > Report at http://europa.eu.int/comm/food/fs/bse/bse42_en.pdf
- > Analytical sensitivity



Data taken from European Commission Report „The Evaluation of Five rapid tests for the diagnosis of TSE“ March 2002

Prionics®-Check PrioSTRIP

DETECTION PRINCIPLE

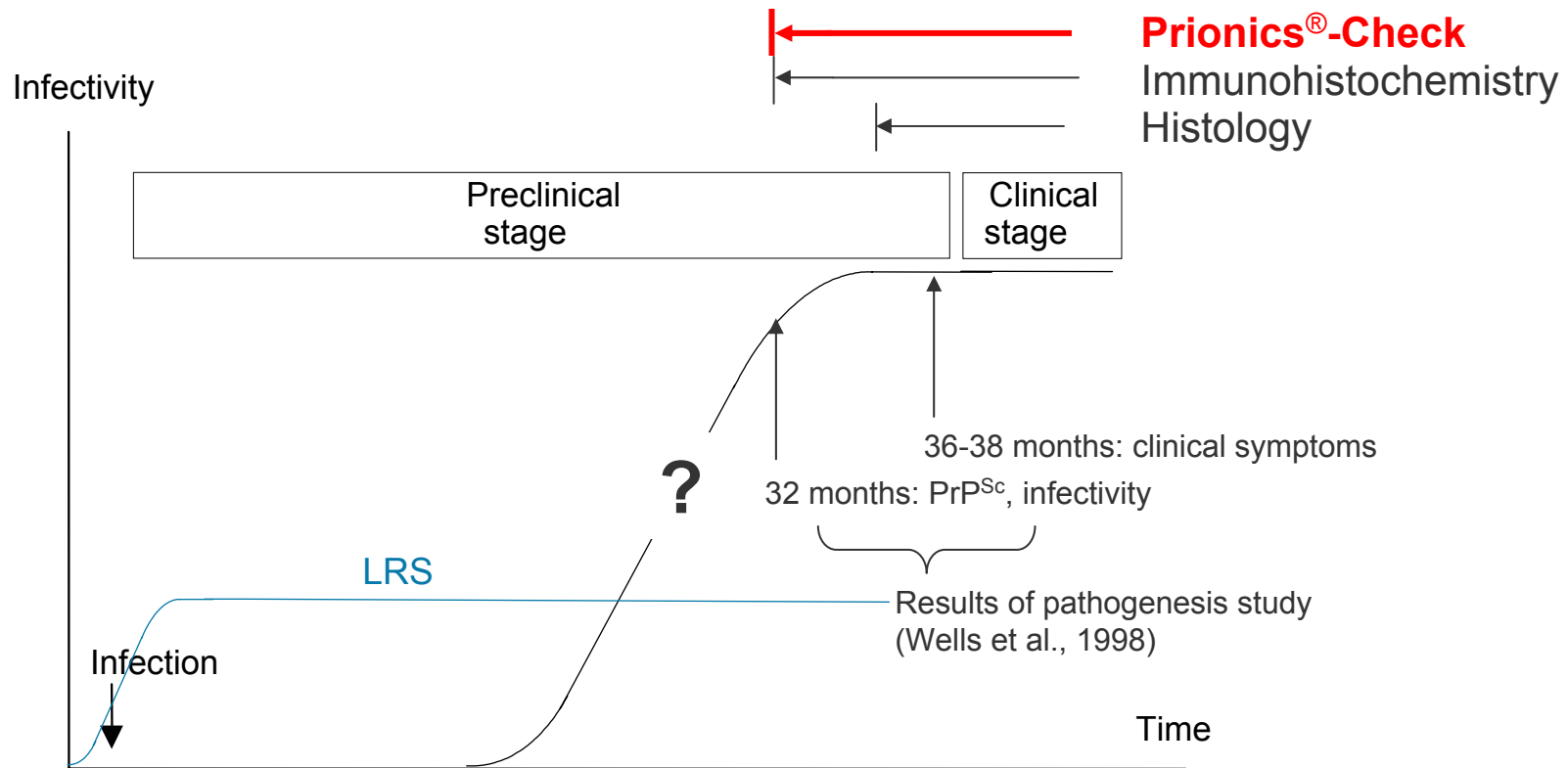


Prionics®-Check PrioSTRIP

SUMMARY

- > Sensitivity and specificity of 100%
 - > More than 4500 negatives and 240 positives correctly identified
- > EU field studies completed
- > Validated in Switzerland
- > High analytical sensitivity in the low picogram range
- > Very rapid kinetics giving results within minutes
- > No need for laborious sample preparation steps such as centrifugation or precipitation and no need for sophisticated laboratory equipment

Prionics®-Check Immunoassay COMPARISON WITH REFERENCE METHODS



In vivo TSE Diagnostics

GOALS

- > Detection of preclinical/subclinical TSE cases
- > Assessment of TSE epidemics (vCJD, BSE, CWD, Scrapie)
- > Safety of blood supply and blood-derived products
- > Safety of pharmaceutical products produced from animal tissues

In vivo TSE DIAGNOSTICS INFECTIVITY/ PrP^{Sc} IN BLOOD

- > TSE infectivity was documented to be present in the blood of experimentally infected rodents and sheep models
- > Blood and blood fractions of CJD patients was occasionally infectious to rodents
- > First vCJD case likely to be caused by blood transfusion
- > **The European CJD surveillance system**
 - > More than 12500 specimens of tonsil and appendix screened
 - > 3 showed PrP^{vCJD}
 - > Potentially 3800 people could be carriers of vCJD in the UK

Detection of PrP^{Sc}

PrP^{Sc} SPECIFIC ANTIBODY 15B3

Prion (PrP^{Sc})-specific epitope defined by a monoclonal antibody

C. Korth*, B. Stierli†, P. Streit‡, M. Moser*, O. Schaller*, R. Fischer‡, W. Schulz-Schaeffer§, H. Kretzschmar§, A. Raeber||, U. Braun†, F. Ehrensperger☆, S. Hornemann#, R. Glockshuber#, R. Riek#, M. Billeter#, K. Wüthrich# & B. Oesch*

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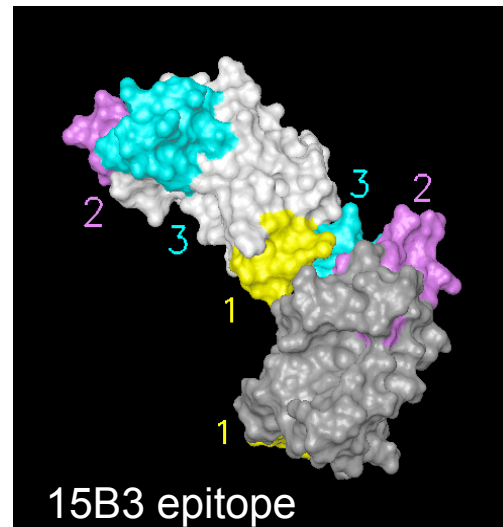
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Prions are infectious particles causing transmissible spongiform encephalopathies (TSEs). They consist, at least in part, of an



Nature 390 (6655) : 74-77, 1997

TSE Diagnostics

DETECTION PRINCIPLES

6H4 antibody

normal



BSE



Prionics®-Check WESTERN
Prionics®-Check LIA
Prionics®-Check PrioSTRIP

15B3 antibody

normal



BSE



Immunoprecipitation
ELISA

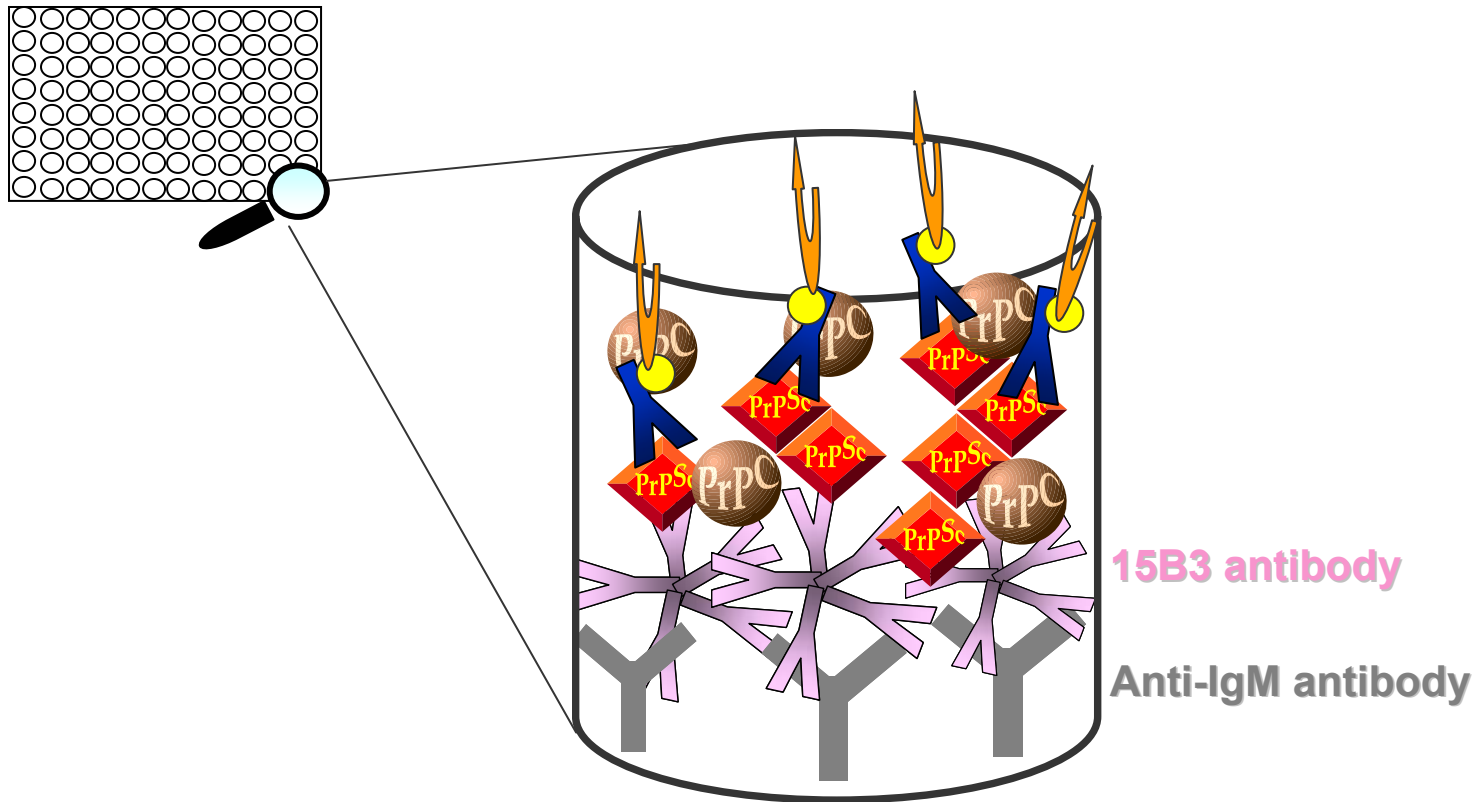
15B3 Immunoprecipitation DETECTION OF PrP^{Sc} AND PrP^{BSE}



Monoclonal antibody 15B3 specifically immunoprecipitates PrP^{Sc} and PrP^{BSE} from brain of TSE infected animals

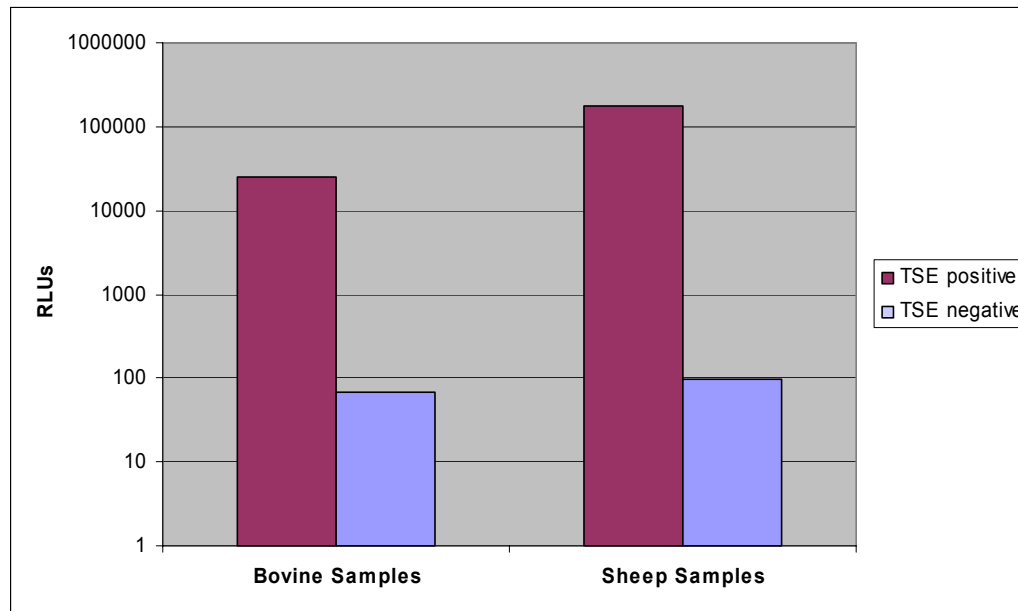
Detection of PrP^{Sc}

ELISA TEST PRINCIPLE



15B3 Immunodetection by ELISA

DETECTION OF PrP^{Sc} IN BRAIN



Monoclonal antibody 15B3 specifically detects PrP^{BSE} and PrP^{Sc} in a Sandwich ELISA

SUMMARY

- > The current TSE diagnostics (Prionics®-Check WESTERN, LIA and PrioSTRIP) are well suited for postmortem detection of prions in cattle, sheep and deer
- > Conformation-specific antibody 15B3 detects only the pathological isoform of PrP in cattle, humans, sheep, and deer
- > Sensitive and specific detection of PrP^{Sc} without protease-digestion in combination with the presented ELISA platform opens the way for early diagnostic testing in peripheral tissues and body fluid